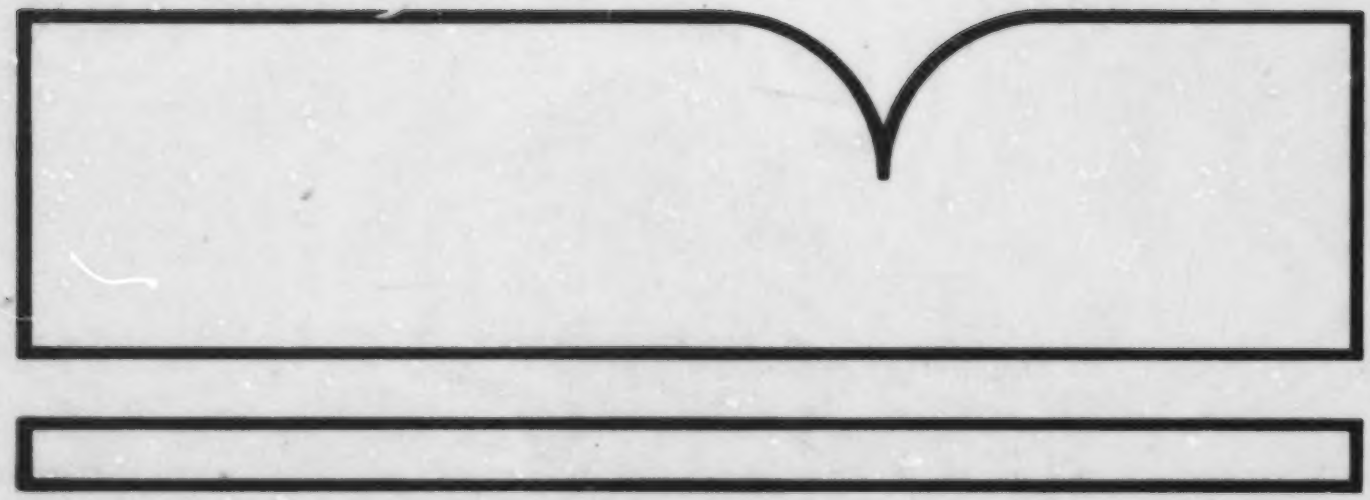
PB82-127473

Biochemical Changes in Humans Upon Exposure to Nitrogen Dioxide While at Rest

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Sep 81



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PB82-127473

TECHNICAL REPORT DATA

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1. REPORT NO. EPA-600/J-80-108	2. JOURNAL ARTICLE
3. RECIPIENT'S ACCESSION NO.	
4. TITLE AND SUBTITLE BIOCHEMICAL CHANGES IN HUMANS UPON EXPOSURE TO NITROGEN DIOXIDE WHILE AT REST	5. REPORT DATE September 1981
	6. PERFORMING ORGANIZATION CODE 308041
7. AUTHOR(S) SUZANNE CHANEY, WENDY BLOMQUIST, PAULETTE DEWITT, AND KEITH MULLER	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Clinical Research Branch Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO. AAGA1D
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development (RTP, NC) Health Effects Reasearch Laboratory US Environmental Protection Agency Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Archives of Environmental Health 36(2): 53-58, 1981

16. ABSTRACT

The biochemical response to controlled exposures of nitrogen dioxide (NO_2) was studied in 19 human subjects exposed to 0.2 ppm NO_2 for 2 hr and compared to 15 control subjects exposed to filtered air for 2 hr. Seven biochemical-blood parameters, including glutathione, red blood cell glutathione reductase, 2,3-diphosphoglycerate, methemoglobin, vitamine E, complement C_3, and IgA were measured prior to exposure, immediately after exposure, and 22 hr following exposure, The only variable that showed significant change due to NO_2 exposure was glutathione. Glutathione is known to protect the erythrocyte from oxidative stress. The increase in glutathione observed upon exposure to NO_2 may be a protective reaction of the erythrocyte to meet the oxidative stress.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA-600/J-80-108
Journal Article

Biochemical Changes in Humans Upon Exposure to Nitrogen Dioxide While at Rest

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ABSTRACT. The biochemical response to controlled exposures of nitrogen dioxide (NO_2) was studied in 19 human subjects exposed to 0.2 ppm NO_2 for 2 hr and compared to 15 control subjects exposed to filtered air for 2 hr. Seven biochemical blood parameters, including glutathione, red blood cell glutathione reductase, 2,3-diphosphoglycerate, methemoglobin, vitamin E, complement C_3, and IgA were measured prior to exposure, immediately after exposure, and 22 hr following exposure. The only variable that showed significant change due to NO_2 exposure was glutathione. Glutathione is known to protect the erythrocyte from oxidative stress. The increase in glutathione observed upon exposure to NO_2 may be a protective reaction of the erythrocyte to meet the oxidative stress.

MEASURES OF atmospheric concentrations of nitrogen dioxide (NO_2), a common air pollutant, have indicated a rising trend over the past 10 yr.[1] Although many studies have examined the effects of NO_2 in animal models, and pulmonary effects in humans, there is little information available at present concerning the biochemical effects of inhaled NO_2 in humans. Nitrogen dioxide animal exposure studies have reported an increase in the development of lung emphysema and cardiovascular disease[2-10] and an increased susceptibility to bacterial and viral infection.[5,11,12]

Casson and Simmons[13] have reported data on in vitro NO_2 exposure studies of human erythrocytes which suggest that NO_2 may influence several loci of erythrocyte metabolism, including accumulation of glycolytic intermediates, oxidation of NADH, NADPH, and glutathione, and alteration of NADH-dependent enzymes.

Information concerning the in vivo effects of inhaled NO_2 at the cellular level in humans is virtually nonexistent. The urban annual average for NO_2 is 0.04 to 0.05 ppm, while for rural areas it is $\leq$ 0.01 ppm. Presently a short-term standard range of 0.1 to 0.5 ppm NO_2 is being considered. An NO_2 concentration of 0.2 ppm was chosen for this initial study to give an indication of possible health effects that may occur as a result of a short-term exposure to high levels of NO_2.

METHODS AND MATERIALS

Subjects. Healthy, nonsmoking, Caucasian male university students participated in the study. Each subject was screened with a physical examination; the Duke University Computer History form and the Minnesota Multiphasic Personality Inventory were also completed. Each participant was advised of any risk involved in the study and signed an informed consent statement with the understanding that withdrawal at any time during the study would be allowed. The group of volunteers was charac-

terized by an average height of 183.1 cm [standard deviation (*SD*) = 6.1 cm; range = 164-195 cm], an average weight of 72.9 kg (*SD* = 76 kg; range 61.2-95.2 kg), and an average age of 23.8 yr (*SD* = 3.2 yr; range = 18.25-29.67 yr). There were 15 subjects in Group 1 (controls) and 19 subjects in Group 2 (experimentals).

Experimental Design. Each subject was exposed once for 2 hr while at rest. The control group was exposed to filtered air and the experimental group was exposed to 0.2 ppm NO_2. The chamber atmosphere was maintained at a temperature of 22°C, a relative humidity of 40%, and an air flow of 227 m^3/min. The 2-hr exposure began and ended at the same times each day. Blood was drawn immediately preceeding and immediately following the 2-hr exposure, and 22 hr post-exposure.

Data were collected in a counterbalanced manner as follows: during week 1, two groups of two subjects each were exposed to NO_2—one group on Monday and the second group on Tuesday. Two groups of two subjects each were exposed to air, one group on Wednesday and the second group on Thursday. The following week the order was reversed. This alternation scheme was continued throughout the experiment and helped control for any day-of-week and any time-of-year effects.

A total of seven serum and red blood cell biochemical parameters were chosen as dependent variables: glutathione (GSH), red blood cell glutathione reductase, 2,3-diphosphoglycerate (2,3-DPG), methemoglobin (Met Hgb), vitamin E, Complement C_3, and IgA.

Statistical Analysis. Multivariate analysis of variance

Table 1.—Summary Statistics for All Seven Dependent Variables*

Hr	Mean	*SD*	Min	Max	Skewness
		Glutathione, mg/100 ml			
0	38.57	6.47	29.23	55.20	0.88
2	38.46	7.21	23.51	59.65	0.95
22	38.54	5.98	25.71	56.39	0.47
		2,3-DPG, μmol/ml RBC			
0	4.13	0.52	3.38	5.96	1.26
2	4.42	0.70	3.08	6.48	0.72
22	4.39	0.45	3.47	5.21	−0.07
		Met Hgb, % total Hgb			
0	0.54	0.14	0.30	1.03	1.74
2	0.53	0.12	0.34	0.87	1.28
22	0.63	0.23	0.37	1.28	1.36
		Vitamin E, μg/ml Serum			
0	5.01	1.30	2.43	7.50	−0.05
2	5.08	1.23	2.52	8.23	0.16
22	4.92	1.08	2.36	7.37	0.20
		Glutathione Reductase, μmol/g Hgb·min			
0	4.41	0.92	2.24	6.26	−0.07
2	4.18	0.97	2.67	7.07	0.78
22	3.93	0.78	2.66	5.83	0.95
		Complement C_3, mg/dl			
0	111.2	27.9	69.0	178.0	0.61
2	112.7	30.9	49.0	180.0	0.47
22	114.4	32.0	61.0	201.0	0.75
		IgA, mg/dl			
0	212.4	107.0	86.0	582.0	1.83
2	217.0	110.5	83.0	593.0	1.73
22	221.6	115.5	97.0	582.0	1.60

*N = 34.

Table 2.—Pearson Correlations Coefficient between Pre-Exposure Measures

	GSH	2, 3-DPG	Met Hgb	Vit E	GSH Reductase	C_3	IgA
GSH	1.0						
2,3-DPG	−0.03	1.0					
Met Hgb	−0.01	−0.07	1.0				
Vit E	−0.19	0.22	−0.10	1.0			
GSH Reductase	0.12	−0.36	0.30	0.09	1.0		
C_3	−0.03	−0.17	0.20	−0.05	0.50	1.0	
IgA	0.23	0.23	0.19	−0.22	0.04	−0.18	1.0

(MANOVAS) was used to test the effects of time of measurement, NO_2 exposure, and the interaction between time and NO_2 exposure. Since one MANOVA was conducted for each of the seven variables, the appropriate significance level is 0.05/7 = 0.0071. For each variable the 0 hr measure was used as a covariate and the 2 hr and 22 hr measures were the dependent variables.

Blood analysis. Glutathione was measured by the method of Patterson and Lozarow.[14, 15] Red blood cell glutathione reductase was assayed by the method of Nichoalds,[16] modified such that the enzymes was preincubated at 0°C for 30 min in the presence of 60 μM FAD, and the reaction was initiated by the simultaneous addition of 2 mM oxidized glutathione (GSSG) and 0.24 mM NADPH. Complement C_3 and IgA were measured using the FIAX™ method of International Diagnostic Technology.[17] Serum vitamin E was measured by the method of Chaney et al.,[18] and 2,3-DPG in erythrocytes by the Nygaard and Rorth[19] method. Methemoglobin was measured by the method of Hegesh et al.[20]

Materials. Manual assays for glutathione reductase, GSH, 2,3-DPG, and Met Hgb were conducted on a Cary 118 spectrophotometer. Reagent kits for 2,3-DPG were purchased from Calbiochem (LaJolla, California). Complement C_3 and IgA reagent kits were purchased from International Diagnostic Technology (Santa Clara, California).

Alloxan monohydrate, D,L-α-tocopherol, GSSG (free acid, grade III), FAD (disodium salt, grade III), NADPH (tetrasodium salt, Type 1), and GSH (98-100% purity) were purchased from Sigma Chemical Company (St. Louis, Missouri). Potassium ferricyanide, sodium cyanide, and glacial acetic acid were purchased from Fisher Scientific Company (Fair Lawn, New Jersey). Silica gel plates for serum vitamin E chromatography were purchased from Bodman Chemicals (Doraville, Georgia).

RESULTS

Table 1 provides summary statistics for all 7 dependent variables. Since these results are combined for both air and NO_2, all results are based on 34 subjects. All values for each variable appear to fall within a "normal" range; only IgA shows any appreciable skewness. In general, these results do not invalidate the use of analysis of variance.

Table 2 shows the correlations between the seven blood variables on the pre-exposure measure. The correlations indicate that, in general, little overlap exists among the seven variables tested. However, GSH reductase does show moderate overlap with the other variables, particularly with complement C_3 and 2,3-DPG. These results indicate that none of the dependent measures is redundant with any of the others.

Table 3 allows evaluation of the use of the pre-exposure measures as covariates. The correlations are low only for complement C_3 and 2,3-DPG. Covariate analysis helped provide a more sensitive statistical text for the other variables. To the extent that this correlation is close to a value of one, then covariate analysis reduces the variability within groups by adjusting for individual differences; therefore, between group differences are increased in units of a smaller standard error (SE).

Table 3.—Pearson Correlations Coefficients between Dependent Variables and Pre-Exposure Measures

	GSH	2,3-DPG	Met Hgb	Vit E	GSH Reductase	C_3	IgA
2 hr	0.66	0.27	0.55	0.82	0.42	0.25	0.93
22 hr	0.56	0.23	0.23	0.68	0.43	0.38	0.93

Table 4.—MANOVA Source Table

Source	F*	Numerator df	Denominator df	P
		Glutathione		
Group, G	9.60	1	31	0.0040†
Time, T	0.97	1	31	0.3309
G X T	0.19	1	21	0.6674
		2,3-DPG		
Group, G	4.38	1	31	0.0445
Time, T	0.39	1	31	0.5350
G X T	1.08	1	31	0.3060
		Met HgB		
Group, G	1.27	1	31	0.2680
Time, T	1.20	1	31	0.2810
G X T	4.48	1	31	0.0424
		Vitamin E		
Group, G	0.03	1	31	0.8589
Time, T	2.77	1	31	0.1062
G X T	0.08	1	31	0.7838
		Glutathione Reductase		
Group, G	0.10	1	31	0.7502
Time, T	0.04	1	31	0.8405
G X T	0.08	1	31	0.7811
		Complement C_3		
Group, G	1.98	1	31	0.1697
Time, T	0.85	1	31	0.3639
G X T	0.90	1	31	0.3498
		IgA		
Group, G	0.02	1	31	0.8795
Time, T	0.05	1	31	0.8159
G X T	0.03	1	31	0.8648

*Exact multivariate *F* test.
†Significant at the .05/7 = .0071 level (Bonferroni corrected for familywise .05 level).

Table 4 summarizes the MANOVA conducted for each dependent variable. Three tests were conducted for each variable. The group test evaluates the differences between air and NO_2 exposure groups, averaged across 2 hr and 22 hr. The time test evaluates differences between the 2 and 22 hr measure, averaged across air and NO_2. The Group by Time interaction test evaluates the difference in trend across time for the two exposure groups.

Only one variable, glutathione, showed a significant difference, ($P < .0071$) between the air and NO_2 groups. The mean GSH level in the air exposure group was 36.8mg/100 ml blood with a SE of 0.80 mg/100 ml blood. For the NO_2 exposure group the mean was 40.1 mg/100 ml blood with a SE of 0.71 mg/100 ml blood. Note that these have been adjusted to the average 0 hr GSH level, and each mean is the average of the 2 hr and 22 hr means.

Table 5 provides estimated means for the air and NO_2 groups at 2 hr, adjusted to the average level of the covariate (pre-exposure measure). These are presented with their standard errors to indicate the adjusted levels and the variability observed.

DISCUSSION

The present study was designed to elucidate the biochemical effects of NO_2 at the cellular levels in humans. Blood parameters that are involved in maintaining cellular reductive detoxification ability and parameters indicative

to correlate response observed with level of NO_2 exposure.

We are presently investigating the effects of 0.5 ppm NO_2 in combination with several sulfur-containing airborne particulates. Once the preliminary exposure studies are completed, more complex exposure protocols will hopefully provide answers to many of the questions that still remain unanswered with regard to the response at the cellular level to an oxidative challenge in man.

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Submitted for publication September 16, 1980; revised; accepted for publication January 6, 1981.

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